MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF SOIL FERTILITY: VII. CARBON DIOXIDE EVOLUTION[1]

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HISTORICAL

The evolution of carbon dioxide has been used by a number of investigators as an index of the decomposition of organic matter in the soil.

Wollny (38) found in 1880 that the carbon dioxide content of the soil rises and falls with the amount of organic matter present in the soil. Kiszling and Fleischer (8) used the production of carbon dioxide in peat soils as an index of the rapidity of the decomposition going on in the soil; the addition of sand was found to stimulate oxidation, while the temperature was among the most important factors affecting oxidation.

Déhérain and Démoussy (2) placed the soil under examination in a closed tube of 100 cc. capacity and kept it at constant temperature. At the end of a certain period of incubation, the gas was extracted and the carbon dioxide present determined. It was found that the formation of carbon dioxide was due almost entirely to the action of microörganisms and that the carbon dioxide content increased with temperature to about 65°C., then decreased, and at 90° another increase took place due to chemical agencies. There is an optimum moisture content for the formation of carbon dioxide, which is also influenced by the state of division of the soil and its aeration. Although sterile soils were found to produce small amounts of carbon dioxide, the latter increased twenty-five times when soil infusion was added [Severin (23)]. Sterilized and inoculated soil gave two to five times as much carbon dioxide as unsterilized and uninoculated soil.

Russell (21) measured the actual amount of oxygen absorbed by the soil as an index of soil oxidation instead of determining the carbon dioxide produced. He found that the rate of absorption of oxygen increased with temperature, the amount of water (up to a certain point) and the amount of calcium carbonate present in the soil. These conditions also increase soil fertility. Russell, therefore, suggested the use of soil oxidation as a measure of fertility. The amount of oxygen absorbed measures the total action of soil microörganisms, which are responsible for the decomposition processes in the soil.

Stoklasa and Ernest (30) placed 1-kgm. portions of sieved soil in glass cylinders through which a current of air was passed at the rate of ten liters in twenty-four hours. They observed that the evolution of carbon dioxide by a soil, under certain conditions of moisture and temperature, in a certain length of time, can furnish a reliable and accurate method for the determination of bacterial activities in the soil; the presence of organic matter and the temperature were found to be of greatest importance. Stoklasa (25, 26) further found that the evolution of carbon dioxide occurs most abundantly in neutral or slightly alkaline soils, abundantly supplied with readily assimilable plant nutrients and well aerated.

The production of carbon dioxide [Stoklasa (27)] was in direct proportion, not to the total carbon content of the soil, but to the available organic matter in the soil. The evolution of

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carbon dioxide was thus found to be an index of the availability of the soil organic matter, or the ease with which it decomposes. Two methods were suggested by Stoklasa (27, 28, 29) to demonstrate this; (1) the determination of the amount of carbon dioxide produced in twenty-four hours by 1 kgm. of soil remoistened after first being air-dried and (2) sterilizing the soil and then inoculating 1 kgm. with 10 gm. of cattle manure extract and determining the amount of carbon dioxide produced in twenty-four hours. The two methods gave the following results:

SOIL TYPE	CARBON CONTENT	CO_2 PRODUCED IN 24 HOURS BY 1 KGM. OF FRESH SOIL	CO_2 PRODUCED IN 24 HOURS BY 1 KGM. OF STERILIZED, INOCULATED SOIL
	per cent	*mgm.*	*mgm.*
Tenacious clay soil	1.68	8.2	14.0
Diluvial loam	2.12	14.6	27.8
Alluvial soil	1.73	36.6	59.8

The amount of carbon dioxide produced was found to depend on the quantity and kind of organic matter, physical and chemical condition of the soil, numbers and kinds of microörganisms. It was found to serve as an index not only of the activities of the microörganisms of the soil but of the amount of readily decomposable organic matter.

Carbon dioxide evolution was thus found to run parallel with numbers of microörganisms and also [Stoklasa (27)] with nitrification in the soil, as shown in the following table:

SOIL DEPTH	UNCULTIVATED, UNFERTILIZED LOAM SOIL		CULTIVATED, FERTILIZED, UNDER CLOVER		MANURED AND FERTILIZED, CULTIVATED UNDER BEETS	
	Bacteria in 1 gm.	CO_2 by 1 kgm. in 24 hours	Bacteria in 1 gm.	CO_2 by 1 kgm. in 24 hours	Bacteria in 1 gm.	CO_2 by 1 kgm. in 24 hours
cm.	*thousands*	*mgm.*	*thousands*	*mgm.*	*thousands*	*mgm.*
10–20	230	16.5	1,800	38.6	4,700	47.5
20–30	256	19.4	2,350	38.8	3,529	49.7
30–50	208	9.8	1,600	20.2	2,100	28.5
50–80	14	3.3	540	6.3	184	6.6
80–100	5	2.1	72	2.7	95	2.3

Van Suchtelen (32) passed a current of air, usually 16 liters in 24 hours, through 6 kgm. of soil placed upon pure sand in a jar. The intensity of carbon dioxide production was found to be much greater at the beginning of the experiment and rapidly decreased after a short while. The amount of carbon dioxide produced was measured until it reached a uniformly low level; the average amounts of carbon dioxide produced per unit time from the different soils served for comparison. He concluded that the determination of carbon dioxide formation by different soils furnishes a better means for estimating the bacterial activities in the soils than the numbers of bacteria. Cultivation, aeration and nutritive salts were found to exert stimulating effects upon carbon dioxide production; moisture and organic matter content of the soil are among the most important factors. In a later contribution, van Suchtelen (33) considered the microbiological activities in the soil from the standpoint of energy. The amount of heat produced by a given soil under laboratory conditions, during a definite interval of time, was taken as a unit of comparison.

Rahn (20) used sugar solutions containing $CaCO_3$ and inoculated with soil; he measured not only the carbon dioxide produced by the microörganisms from the sugar but also that formed from the interaction of organic acids with $CaCO_3$. The use of carbon dioxide production in soil as a measure of soil fertility was also suggested by König, Hasenbäumer and Glenk (11), who measured the carbon dioxide evolved from 1 kgm of soil with and without the addition of 1 gm. of glucose or urea.

Russell and Appleyard (22) found the curves for bacterial numbers, nitrate and carbon dioxide content of the soil air sufficiently similar to justify the view that all these phenomena are related. A rise in bacterial numbers was accompanied by a rise in the carbon dioxide content in the soil air and somewhat later by a rise of nitrate in the soil. The rate of decomposition of organic matter in the soil was, therefore, looked upon as a function of bacterial activity. The rate of biochemical activities in the soil was found to attain maxima in late spring and in autumn, and minima in summer and winter. In autumn the bacteria increased first, then the carbon dioxide content rose, and finally the nitrate increased.

Most of the early work and considerable later work on carbon dioxide in soils has been carried out with the gases taken from the soils *in situ*. A large amount of such work seems to demonstrate the difficulties of obtaining indicative results by analyzing the atmosphere of field soils in place.

Pettenkofer (18) suggested a method for measuring the carbon dioxide produced by soils. He aerated soils with carbon dioxide free air through a container and measured the carbon dioxide in the outgoing air.

Petersen used a similar apparatus (17) and since then the Pettenkofer method has been used extensively by numerous investigators with various modifications. Among these might be mentioned Wollny (39) who aerated the soil mixed with sand, Déhérain and Démoussy (2) Stoklasa and Ernest (30), van Suchtelen (32), Lemmermann, et al. (13), Klein (9), and Gainey (6).

Russell (21), and Darbishire and Russell (1) used oxygen consumption, to measure soil oxidation processes. Leather (12) extracted the gases from the soil samples by suction and subsequently analyzed them for carbon dioxide.

Few of these methods have been used to any extent recently. The method commonly used at present is to measure the carbon dioxide in air, previously freed from carbon dioxide, passed continuously over the surface of soil placed in containers. Under these conditions the soil more nearly approaches normal conditions than where it is aerated, which greatly accelerates microbiological activities. This method has been used by Fred and Hart (5), Fraps (4). Potter and Snyder (19), Merkle (14), Neller (15) and has proved satisfactory in our hands.

Potter and Snyder (19) state that the amount of air passing over the soil in the laboratory does not materially affect the amount of carbon dioxide evolved. The addition of moisture to an air-dry soil was found to result in a rapid increase in the amount of carbon dioxide evolved, followed by a gradual drop. Previous drying of the soil alters its colloidal condition, permitting an increased rate of oxidation. It also alters the chemical condition of the organic matter making it more readily available for the activities of microörganisms. Similar results were obtained by Klein (9) and others.

Neller (16) determined the carbon dioxide producing capacity of the soil by placing tumblers containing 200-gm. portions of soil, to which 0.75 gm. of soybean hay had been added, under bell jars, through which carbon dioxide free air was passed for sixteen days. On comparing two limed and two unlimed soils, he obtained distinct correlations between crop yield, nitrate accumulating and numbers of bacteria, but these did not correlate with ammonia accumulation, as shown below:

PLOT NUMBER	CROP YIELD (10 YEARS)	CO_2 PRODUCTION	NO_3-N* ACCUMULATION	NH_3-N* ACCUMULATION	BACTERIAL NUMBERS
	lbs.	*mgm.*	*mgm.*	*mgm.*	*millions*
11A (acid)	2282.4	353.4	10.3	11.93	2.5
11B (limed)	2928.0	505.6	22.3	11.44	6.2
21 (acid)	2015.8	389.9	16.1	11.56	5.1
24 (limed)	2661.1	542.0	33.9	11.68	6.5

* From 100 mgm. nitrogen in the form of dried blood.

König and Hasenbäumer (10) also found that carbon dioxide formation goes hand in hand with bacterial numbers. Gainey (6) observed the parallel formation of carbon dioxide, ammonia and nitrate from organic substances rich in nitrogen (cottonseed meal and dried blood), when moisture and aeration were favorable; the correlation was especially noticeable between the ammonia and carbon dioxide production.

The discrepancy between Neller's and Gainey's results can be readily explained by a consideration of the carbon and nitrogen metabolism of the microörganisms concerned, especially the fungi. It will be shown elsewhere that the relative amounts of carbon dioxide and ammonia formation from any organic substance depends upon the metabolism of the particular organism and the carbon-nitrogen ratio of the organic material. For every unit of carbon assimilated by the organism, as well as for the carbon dioxide formed, there is a definite amount of nitrogen assimilated. When the organic matter (dried blood or cottonseed meal) contains more nitrogen than the organism needs for metabolic processes, a part of the nitrogen will be left as a waste product in the form of ammonia; when the organic matter contains less nitrogen than the organism requires, there will be no ammonia accumulation and the carbon compound will be decomposed only so far as the nitrogen supply, whether present in the material or added in inorganic forms, will permit.

Gainey determined the carbon dioxide formation and ammonia accumulation from nitrogen-rich organic materials, such as dried blood or cottonseed meal. It would be expected that both would run parallel, since for every unit of carbon used up, either for structural or energy purposes, there is a corresponding amount of nitrogen liberated, as ammonia, in excess of that required by the organism. Neller, however, determined carbon dioxide production from soybean meal, a substance comparatively low in nitrogen, and ammonia formation from dried blood. The rate of decomposition of these two substances in the same soil, as indicated by the evolution of carbon dioxide, is different, as shown by Starkey (24).

It is also important to point out that the formation of carbon dioxide in the soil depends not only upon the absolute carbon content of the soil, but upon the ease of its decomposition, as shown by Stoklasa (27) and Gehring (7).

A brief review of the literature, therefore, tends to indicate that the evolution of carbon dioxide is a good index of decomposition of organic matter, of microbial activities and of soil fertility. The fact that the numbers of microörganisms and the nitrifying capacity of soil have been found in previous investigations (34, 36), to be good indices of soil productivity and the fact that Stoklasa (28, 29), Russell and Appleyard (22), Neller (15), van Suchtelen (32) and others found that evolution of carbon dioxide runs parallel to bacterial numbers and to the nitrifying capacity of the soil, tend to emphasize further that we are dealing here with an important soil microbiological process, which may serve as a proper index for a microbiological analysis of the soil.

METHODS

In studying the power of the soil to decompose organic matter, we often use the terms "oxidative capacity," "carbon dioxide producing capacity," "capacity for decomposing organic matter," "respiratory capacity of soils," etc., all of which mean about the same, namely the decomposition of organic matter in the soil by microörganisms, whereby energy is liberated. Carbon dioxide may be formed not only as a result of oxidation, but as a result of hydrolysis, as in the case of formation of alcohol and carbon dioxide from dextrose.

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$$

On the other hand, energy may be liberated without the formation of carbon dioxide, as in the case of the anaerobic transformation of dextrose to lactic or acetic acids.

$$C_6H_{12}O_6 = 2CH_3CHOH \cdot COOH \ (+ 15 \text{ Cal.})$$
$$C_6H_{12}O_6 = 3CH_3 \cdot COOH \ (+34 \text{ Cal.})$$

Some carbon dioxide undoubtedly also originates in normal soils from carbonates interacting with organic or mineral acids by biological agencies.

The absorption of oxygen would also be only a partial index of energy transformation, since some energy is liberated without the intervention of free oxygen. It would, therefore, be more accurate to use the calorific value of the soil, or liberation of heat as a result of the activities of microörganisms, as an index of energy transformation, as suggested by van Suchtelen (33). However, in view of the complex apparatus necessary for the determination of the latter and in view of the fact that the carbon dioxide is a final product of energy utilization by the majority of heterotrophic soil microörganisms, while only a small amount of it is reassimilated by the autotrophic bacteria, we may use the evolution of carbon dioxide as an index of respiration of soil microörganisms without danger of introducing appreciable errors.

A differentiation should be made between the "respiratory power of the soil" itself and the "decomposing power of the soil" or its ability to decompose added organic matter. Respiratory power is measured by the carbon dioxide produced (from the soil itself), when a definite amount of soil is placed under optimum conditions of moisture and temperature. This depends upon:

1. The number and kind of microörganisms present. 2. The amount of organic matter in the soil. 3. The composition of this organic matter (the degree of its decomposition). 4. Soil aeration. 5. Moisture content. 6. Physical condition of the soil. 7. Chemical composition (altered by fertilization). 8. Soil reaction. 9. Kinds of plants grown (Stoklasa, 25).

Decomposing power is measured by the rate at which a soil decomposes organic matter added to it. It is influenced by most of the factors mentioned above, particularly by the microbial population and the physical and chemical conditions of the soil. Furthermore, a difference in the composition of the organic matter used will effect a change in the activity of the various groups of soil microörganisms and different soil constituents may become limiting factors, e.g., nitrogen or phosphorus, if the added organic matter is low in these.

The amount of carbon dioxide produced in sterilized soils inoculated with a strong cellulose-decomposing organism, preferably a rapidly growing fungus may also be measured. This is preferable to the use of manure, as done by Stoklasa, since no additional nutrients are added with a pure culture.

The "respiratory power of the soil," which indicates the condition of the organic matter in the soil, its ease of decomposition or its availability, when the soil is brought under favorable temperature and moisture conditions, as

well as activities of microörganisms in the given soil or their respiration intensity can be determined in three different ways:

1. One-kilogram portions of fresh soil, from a composite sample taken to a depth of 6½ inches and put through a 3-mm. sieve, are placed in pots. Enough water is then added to bring the moisture content of the soil to the optimum. The pots of soil are then placed in the respirator and the amount of carbon dioxide evolved determined at various intervals for seven to fourteen days. This method has been used in our investigations.

2. One-kilogram portions of air-dried, sieved soil, taken to a definite depth, are placed in proper containers, adding the necessary amount of water, and the carbon dioxide evolved in forty-eight hours is determined. Stoklasa (28, 29), using only a twenty-four hour period, found that an infertile soil, poor in organic matter, will produce 8–14 mgm. carbon dioxide, a good beet soil produces 56–68 mgm., and a medium soil about 30 mgm.

3. One-hundred gram portions of fresh soil, prepared as for method 1, are placed in 300-cc. flasks with long necks (*A* in fig. 1). Cotton plugs are placed in the necks of the flasks and in the glass connections. After the proper amount of water is added (50 per cent of total moisture-holding capacity), the flasks are sterilized for 1–1½ hours, on two consecutive days, at 15 pounds pressure. The soils are then inoculated with a culture of a common green *Trichoderma* which was found to be one of the most active groups of soil fungi decomposing celluloses, proteins, pectins and other complex organic substances. The flasks are then connected with the $Ba(OH)_2$ tubes in the respirator and the amount of carbon dioxide evolved is determined for 12–14 days. This method has not been used extensively in the following experiments, but the results obtained are very indicative. Two soils, 5A a fertile soil rich in organic matter, and 7A an infertile soil, poor in organic matter were compared. By this method, 124.08 mgm. and 37.40 mgm. of carbon dioxide respectively were found to be given off in eight days.

The "decomposing power of the soil" can be determined by a group of methods, which differ chiefly in the kind of organic matter added to the soil. A few substances are suggested here, since their decomposition is directly influenced not only by the microbiological activities in the soil, but also by its chemical composition, the presence of available nitrogenous substances and to a lesser extent of phosphates.

1. *Dextrose.* This substance is very readily decomposed in the soil and an excess of material as well as a long period of incubation may obliterate finer differences in the activities of the microörganisms in the different soils. Five hundred milligrams of dextrose was added to 100 gm. of soil. The carbon dioxide evolved was determined every six or twelve hours for a period of 48–72 hours and curves were obtained, which bring out distinctly the differences in the microbiological activities of the different soils. Since dextrose is used very readily as a source of energy not only by the soil fungi and actinomycetes, but also by the great majority of heterotrophic soil bacteria, including the nitrogen-fixing organisms, the rate of decomposition is very rapid. The utilization, by the soil organisms, of all the nitrogen available, during the decomposition of dextrose, will not necessarily stop the production of carbon dioxide. If the nitrogen-fixing organisms are at all active in the soil, they will tend to obtain nitrogen from the atmosphere. In case these organisms fail to develop rapidly and the small amount of nitrogen available is used up, the depressing effect of the limited amount of nitrogen will be registered in the decrease in carbon dioxide production.

2. *Cellulose.* The carbon dioxide evolved from 1 gm. of cellulose added to 100 gm. of soil gives information not only on the "decomposing power of the soil" but also on the amount of available nitrogen and phosphate present in the soil. This is due to the fact that the cellu-

lose is decomposed in the soil (with the exception of alkaline or partially sterilized soils) primarily by fungi. These rapidly growing organisms consume a great deal of nitrogen in the synthesis of their mycelium and it soon becomes a limiting factor. The whole question of cellulose decomposition in the soil will be discussed in detail in the following paper of this series. The distinctive difference in the curves of carbon dioxide evolution from dextrose and cellulose has been pointed out by Dvórák (3).

3. *Rye straw and alfalfa meal.* One per cent of alfalfa meal has been used extensively in our studies as reported below. Ordinarily 200 gm. portions of soil were incubated with the organic matter for a period of fourteen days. Rye straw contains about 0.5 per cent of nitrogen and alfalfa meal about 2.5–3.0 per cent, hence the available nitrogen in the soil may become a limiting factor in the first case, but probably will not in the second.

4. *Dried blood.* The use of one per cent of dried blood or other organic material rich in nitrogen, such as casein, permits the determination of the "protein-decomposing" capacity of the different soils. Measurement of ammonia accumulation was not found to be a reliable index of decomposition of organic matter for reasons pointed out elsewhere (35). Ammonia

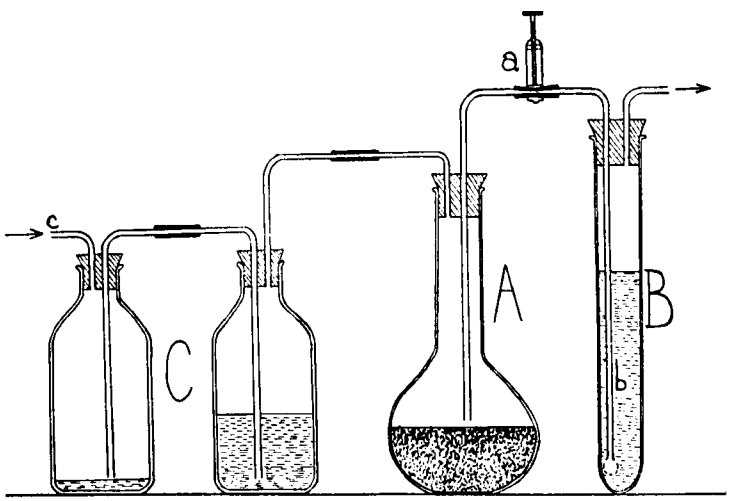

FIG. 1. SMALL APPARATUS FOR DETERMINING THE DECOMPOSING POWER OF SOILS

is an intermediate product in the nitrogen metabolism of a number of organisms and a waste product in the energy metabolism with proteins as a source of energy. It is, therefore, subject to various changes. Carbon dioxide, however, is a final product in the energy utilization. That the rates of ammonia and carbon dioxide formation from proteins are very similar is shown by Gainey (6).

APPARATUS

The apparatus which was used to determine the carbon dioxide evolved from the soils in most of the work was not essentially different from that described by Neller (15, 16). The pots of soil were sealed under bell-jars with paraffin. Air, freed from carbon dioxide by passage through soda lime and through bottles of 10 per cent sulfuric acid, was then drawn through the bell-jars. From there the air was drawn through a modified Truog absorption tower (31) containing 50 cc. standard 0.25 *N* barium hydroxide. The stream of air was drawn through the apparatus continuously at the rate of three liters per hour for the duration of the experiment except for the intervals when the solutions were titrated.

The source of the suction was a water pump described by Neller (15) which gave a steady uniform suction. The apparatus consisted of two sections: one of six and one of seven units. The excess barium hydroxide was titrated back periodically with 0.25 *N* oxalic acid to determine the carbon dioxide absorbed, using phenolphthalein as an indicator. The towers were then renewed. One unit of the apparatus was always blank and was used as a control on the appara tus. Any neutralization of the $Ba(OH)_2$ in the tower connected with this unit was considered to be due to the manipulation and at each titration period allowances were made for this control on all titrations of the $Ba(OH)_2$ from the units containing soils. Ordinarily only very slight corrections were necessary. Six differently treated soils were run in duplicate at one time.

A smaller apparatus was devised for determining the decomposing power of soils. Part of one unit is illustrated in figure 1. The long-neck, flat-bottom flask *A* of 300-cc. capacity took the place of the respiration chamber which in the apparatus described was a bell-jar mounted on a wooden base. The Truog absorption tower was replaced by the 100-cc. test tube *B*. The bulb at the end of tubing *b* was perforated with numerous small holes to break up the bubbles of gas. The air, freed from carbon dioxide by passage through soda lime, was distributed to the various units at *c* and then entered the traps *C*, containing 10 per cent sulfuric acid. This solution prevented diffusion of the gas from one unit to another. The tubing in the two bottles is so arranged as to keep the solution in the traps in the event of back pressure. The air passed over the soil in the respiration chamber and then through the solution in *B* which absorbed the carbon dioxide. From *B* the tubing led to the constant-level siphon water pump.

The respiration apparatus in all cases was enclosed in an incubator room at 25–28°C. The smaller apparatus is less cumbersome than the other and many more units can be run without occupying as much space as the large apparatus.

SOILS USED

Plots of soil from the nitrogen series which have been fertilized alike for fifteen years and used in the previous studies of microbiological methods have also been used in these experiments. A careful record has been kept of the fertilizer applied to the various soils and the resulting crop yields. Although the numbers of microörganisms and nitrifrying capacity of the same soils has been reported previously, the results obtained at the time of sampling for the study of evolution of carbon dioxide are also reported here in order to have a basis for comparison. Ten to twenty-five samples were composited from each plot and put through a 3-mm. sieve.

For the main series of experiments, 1-kgm. portions were placed in glazed earthenware pots of 1-liter capacity. Enough water was added to bring the moisture to optimum which was 50 per cent of the total moisture-holding capacity. To test the decomposing power of the soil, 1 gm. of alfalfa meal was thoroughly incorporated with 200 gm. of soil and enclosed under the belljar respirators in tumblers. The carbon dioxide production from soils treated as in these two cases was determined for 14-day periods.

In determining the production of carbon dioxide by means of the small apparatus, 100-gm. of soil was placed in the flask *A* and 500 mgm. of dextrose was added in solution and well mixed into the soil. The amount of solution added was sufficient to bring the soil moisture content to optimum. When dextrose was used the production of carbon dioxide was measured at six-hour or twelve-hour intervals for 48–72 hours.

With the large apparatus only duplicate determinations were made with each soil. It was found, however, that the results checked up very well. When some organic matter (alfalfa meal) was added for the study of the decomposing power of the soil, discrepancies were often obtained between duplicate determinations, due probably to the uneven distribution of the added material. The simplified apparatus will permit the making of more than 2 determinations for each soil.

Results

The treatment of the plots, crop yields,[2] nitrfying capacity and numbers of microörganisms are given in table 6. The results on the respiratory power of the soil and on the decomposition of alfalfa meal and dextrose are given in tables 1–5.

The annual fertilizer applications per acre made to the soils used in these experiments are as follows:

PLOT NUMBER	FERTILIZER TREATMENT
5A,* 5B	Minerals,† 16 tons cow manure
6A	Minerals, 16 tons horse manure
7A, 7B	Untreated
9A	Minerals, 320 pounds $NaNO_3$
11A, 11B	Minerals, $(NH_4)_2SO_4$ equivalent to 320 pounds of $NaNO_3$
19A	Minerals only

* The plots marked A are unlimed; those marked B receive two tons of ground limestone every 5 years.

† Minerals = 640 pounds acid phosphate and 320 pounds of muriate of potash per acre.

The results on the respiratory power of the soil as determined by our own method, namely from one kilogram of fresh sieved soil, brought to optimum moisture and incubated for fourteen days, are given in table 1. The respiratory power of the soil, by the same method, but only during the first forty-eight hours of incubation is given in table 2. The results presented in table 2 are comparable with those of Stoklasa, who allowed the soil to air-dry, then added moisture and determined the evolution of carbon dioxide in twenty-four hours. In our experiments, fresh sieved soil was used, since it was found that air-drying produces decided physical, chemical and biological changes in the soil. A two-day period of incubation is preferable since twenty-four hours may not be, in some cases, sufficient to free the chamber from all the carbon dioxide.

The results obtained from the two- and fourteen-day periods of incubation are quite comparable. The manured soils (5A, 6A, 5B) lead by far in the

[2] The authors take this opportunity to thank Dr. J. G. Lipman and Prof. A. W. Blair for their kind permission to use soils from these plots and also certain unpublished data on crop yields from these soils for 1923.

amount of carbon dioxide formed, which we would naturally expect, since these soils are much richer in organic matter than the other soils, as indicated by their carbon and nitrogen content (table 6). The limed soils (5B) produced somewhat more carbon dioxide than the corresponding unlimed soil, which would tend to confirm the various observations that lime stimulates the decomposition of organic matter in the soil. Nearly one hundred milligrams of carbon dioxide were given off by the unlimed and more than one

TABLE 1

Respiratory power of soils

PLOT NUMBER	CO_2 PRODUCED FROM 1 KGM. SOIL IN 14 DAYS, BEGINNING AT VARIOUS DATES					AVERAGES
	4-11-22	7-10-22	8-18-22	4-3-23	6-26-23	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
5A	1327.07		855.81	1159.96	653.23	999.02
6A		1100.75	1160.37			1130.56
7A	155.60	290.16	254.01	244.04	260.02	240.77
9A			499.05	459.41		479.23
11A	551.98	356.15	358.65	354.48	470.26	418.30
19A			423.27			423.27
5B	1425.28				774.85	1100.07
7B	443.96	337.38		517.95	655.16	488.61
11B	666.37	387.60		459.42	578.94	523.08

TABLE 2

Respiratory power of soils

PLOT NUMBER	CO_2 PRODUCED FROM 1 KGM. SOIL IN 48 HOURS, BEGINNING AT VARIOUS DATES					AVERAGES
	4-11-22	7-10-22	8-18-22	4-3-23	6-26-27	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
5A	237.24		175.17	206.52	169.91	197.21
6A		182.86	210.82			196.84
7A	21.29	46.93	51.43	36.96	62.33	43.79
9A			122.14	88.27		105.21
11A	74.11	71.25	71.77	58.69	113.23	77.81
19A			90.32			90.32
5B	279.84				215.03	247.44
7B	80.63	66.24		95.65	201.56	111.02
11B	113.42	69.39		79.42	193.30	113.88

hundred milligrams by the limed soil in twenty-four hours. This is more than Stoklasa obtained (68–76 mgm.) for his most fertile sugar beet soils.

The lowest amount of carbon dioxide was produced by the unmanured, unfertilized and unlimed soil 7A, both in two days and in fourteen days. About 22 mgm. of carbon dioxide was given off the first day, which makes it somewhat more than what Stoklasa found in the case of very poor soils. The crop yields reported in table 6 will substantiate the fact that this is the

poorest soil while 5A, 6A and 5B are the most fertile soils in the series. It is important to note the very interesting correlation not only between the respiratory power of these soils with crop productivity but also with numbers of bacteria and nitrifying capacity, as reported in detail elsewhere (34, 36) and as shown in table 6.

Plot 11A has received yearly application of minerals and ammonium sulfate and that has become so acid that it does not support good crop growth; this plot showed a somewhat greater respiratory power than plot 7A. Here again, both two- and fourteen-day periods give comparable results and there is a definite correlation between the respiratory power and crop productivity. The plot receiving minerals only (19A), without any nitrogenous fertilizer or lime comes next to the ammonium sulfate plot in the respiratory power, but is higher in crop yield. This is possibly due to the fact that 11A supports a very abundant fungous flora, while conditions are not favorable for the growth of higher plants. The respiration of the fungi as well as the abundant growth of *acetosella* on 11A probably accounts for the somewhat greater evolution of carbon dioxide than would correspond to its crop production.

The plot receiving sodium nitrate and minerals (9A), the plot receiving lime only (7B) and the one receiving ammonium sulfate, minerals and lime (11B) follow in increasing order of their respiratory capacity. These plots merely show a general parallelism between the respiratory power, crop growth and other biological activities, but not as perfect as in the case of 5A or 7A. This is due to the interfering influence of liming. The addition of lime to an acid soil makes conditions more favorable for the activities of microorganisms, thus resulting in an increase in the numbers of bacteria (decrease of fungi) and an increase in the respiratory power of the soil. This is also accompanied by a greater liberation of plant food and increased crop yield. However, the two may not necessarily correspond, i.e. conditions may be made more favorable for the growth of microörganisms than for the growth of plants. This accounted, in the nitrification studies, for the greater stimulus of lime application to nitrification than to the growth of timothy. The respiratory power is increased somewhat less than the nitrifying capacity so that the results on the respiratory power both in unlimed and limed soils show a closer parallelism with crop yields.

The results on the decomposing power of the soil, when alfalfa is used as a source of organic matter are given in table 3. Here also, decided differences in the capacity of the soils to produce carbon dioxide correspond to their fertility; however, there is no pronounced parallelism. Alfalfa is decomposed in the soil by various groups of microörganisms, especially by fungi. The two poorest soils, 7A and 11A, are distinctly acid in reaction and have, probably as a result of that, an abundant fungous flora, especially 11A. When alfalfa is added to the soil, the fungi rapidly attack the fresh organic matter and a great deal of carbon dioxide is evolved. This will in part compensate for the otherwise lower microbiological activities of these plots in

comparison with the more fertile plots, especially those receiving manure or lime. For this reason, the power of the soil to decompose alfalfa is not considered a valuable index to the respiratory power, and the results are, therefore, not included in the summary shown by table (6).

Decomposition of dextrose as measured by the evolution of carbon dioxide gave results comparable with those obtained in the study of the respiratory power of the soil. However, the length of the incubation period should be short.

TABLE 3

Decomposing power of soils

PLOT NUMBER	CO_2 PRODUCED IN 7 DAYS, BEGINNING AT VARIOUS DATES					AVERAGES
	4-26-22*	7-24-22*	9-1-22*	4-17-23†	7-11-23†	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
5A	706.22		601.72	924.32	782.02	753.57
6A		596.84	613.69			605.27
7A	419.14	431.21	455.27	628.11	586.03	503.95
9A			589.82	684.91		637.37
11A	477.59	453.28	467.47	579.70	622.42	520.09
19A			559.04			559.04
5B	621.51				874.46	747.99
6B		603.84				603.84
7B	490.42	539.62		714.77	807.06	637.97
11B	474.13	546.69		699.43	802.10	630.59

* 1 gm. alfalfa meal added to 200 gm. soil.

† 1 gm. alfalfa meal added to 1 kgm. soil.

TABLE 4

Course of evolution of CO_2 from dextrose

PLOT NUMBER	PRODUCTION OF CO_2 IN 200 GM. SOIL TREATED WITH 0.5 GM. DEXTROSE, AFTER VARYING PERIODS OF INCUBATION			
	24 hours	48 hours	72 hours	96 hours
	mgm.	*mgm.*	*mgm.*	*mgm.*
5A	239.03	328.45	376.78	407.17
7A	53.83	147.88	260.45	327.01
7B	139.48	287.93	362.30	389.79

The carbon dioxide produced from 500 mgm. of dextrose added to 200-gm. portions of three soils of distinctly different fertility (5A, 7A and 7B) may serve as an index for differentiating these soils after 24, and even 48 hours of incubation (table 4). On prolonging the period of incubation, the differences gradually disappear. This is again due to the fact that all soils harbor organisms capable of decomposing dextrose, which organisms develop abundantly in all soils with prolonged incubation. This method depends upon the fact that the soil supporting the most abundant microbiological flora before treatment will effect the most rapid decomposition of the dextrose, particularly

during the first two days after its addition. Table 4 indicates the desirability of using a short period of incubation (24–36 hours) when dextrose is used. For this experiment the soils were sampled in the spring. In midsummer and in the fall, the same method indicated diminished production of carbon dioxide from dextrose per unit time, as shown in table 5. Whether this is due to changes in the physical and chemical conditions of the soils or merely their normal variability is not clear. The production of carbon dioxide for forty-eight hours seemed to bring out the greatest differences between the soils.

The amounts of carbon dioxide evolved from the soils from dextrose (decomposing power) are somewhat parallel to the carbon dioxide produced from the soils themselves (respiratory power), as well as to the crop productions

TABLE 5

CO_2 production from soils treated with dextrose

PLOT NUMBER	AMOUNT PRODUCED IN 48 HOURS FROM 100 GM. SOIL TREATED WITH 500 MGM. DEXTROSE			AVERAGES
	7-18-23	9-12-23	10-17-23	
	mgm.	*mgm.*	*mgm.*	*mgm.*
5A	248.76	162.39	175.65	195.60
7A	64.81	31.63	45.15	47.20
9A		91.99	78.60	85.30
11A	190.96		130.73	160.85
5B	304.19	215.20	159.67	226.35
7B	167.58	98.32	112.26	126.05
11B	184.37	96.94	113.30	131.54

and other biological activities (table 6). The two manured soils were most active, the limed (5B) more so than the unlimed (5A). The unmanured and unlimed soil (7A) was least active, and the soils receiving artificial fertilizers and lime were intermediate. Soils 11A and 9A were tested only twice, and, in view of the fact that the actual amounts of carbon dioxide in the different periods of examination were different, a comparison of the averages of the results from 11A and 9A, on the one hand, with the general averages of the rest of the soils, on the other, might not be justified. For this reason, the carbon dioxide production of these two soils from dextrose are not included in figure 2. The relatively larger amount of available nitrogen in soil 11A brought about by the yearly addition of $(NH_4)_2SO_4$, which is neither used up by the plants nor readily nitrified, as well as the great abundance of fungi probably account for the relative active carbon dioxide production from dextrose in this soil.

Comparisons between crop yields, numbers of microörganisms, nitrifying capacities, respiratory capacities and decomposing capacities of the different soils are given in table 6, and they are graphically represented in figure 4.

The courses of carbon dioxide evolution from 1 kgm. of the soil itself, and in the presence of added organic matter in the form of alfalfa meal based on the average of several different determinations, are given in figure 3.

In plotting figure 4, comparative numbers are used and the data are calculated with the highest figure in each set of determinations being taken as 100. In interpreting these results, it should be kept in mind that a microbiological analysis of a soil would indicate its present crop producing power without further fertilization. It should also give information as to the need of the soil for certain specific fertilizers, organic matter or lime. Of the soils studied,

TABLE 6

Chemical and biological conditions of the soils and their crop productions

PLOT NUMBER	TREATMENT	REACTION OF SOIL	NITROGEN CONTENT	CARBON CONTENT	TOTAL CROP YIELD PER ACRE		NUMBERS OF MICROÖRGANISMS PER GRAM	NITRIFYING CAPACITY† (NO_3-N IN 100 GM. OF SOIL)	CO_2-PRODUCING CAPACITY	
					1908–1922	1923 (Corn)			Respiratory (from 1 kgm. of soil in 14 days)	Decomposing (from 200 gm. of soil + 0.5 gm. dextrose in 48 hours)
		pH	*per cent*	*per cent*	*lbs.*	*lbs.*	*thousands*	*mgm.*	*mgm.*	*mgm.*
5A	Minerals + manure	5.5	0.1463	1.73	69,300	6,108	13,040	8.86	999.02	195.60
7A	Untreated	4.9	0.0826	0.96	15,464	1,710	5,600	2.08	240.77	47.20
9A	Minerals + $NaNO_3$	5.8	0.0994	1.17	57,968	5,273	9,600	6.62	479.23	85.30
11A	Minerals + $(NH_4)_2SO_4$	4.4	0.1064	1.23	41,754	1,753	5,300	2.16	418.30	160.85
5B	Minerals + manure + lime	6.7	0.1428	1.74	59,754	6,478*	12,500	12.07	1100.07	226.35
7B	Lime only	6.5	0.0868	1.18	30,160	5,566	9,800	7.87	488.61	126.05
11B	Minerals + $(NH_4)_2SO_4$ + lime	6.1	0.0952	1.10	61,906	6,440*	10,600	8.75	523.08	131.54

* Actual yield of corn grain was less in 5B than in 5A and was about the same in 11B as in 5A.

† See Waksman (36).

5A and 5B are seen to be the most fertile and 7A the least fertile. The actual crop production of these is actually correlated with the results obtained from a microbiological analysis. Soil 5A produced a larger crop yield during the 15-year period than 5B, but the yield of corn in 1923 was higher in 5B than in 5A. The numbers of bacteria are higher in 5A than in 5B, while the respiratory and decomposing powers, especially the latter, are higher in 5B; the nitrifying capacity of 5B is even still higher than of 5A. The reaction of 5B which is probably more favorable for the activities of the nitrifying bacteria and nitrogen fixing bacteria, is probably responsible for these differences.

The data for 7A are quite parallel, except for the numbers of microörganisms which appear higher than the other data. Here again, nitrification, crop yield, respiratory and decomposing powers are correlated.

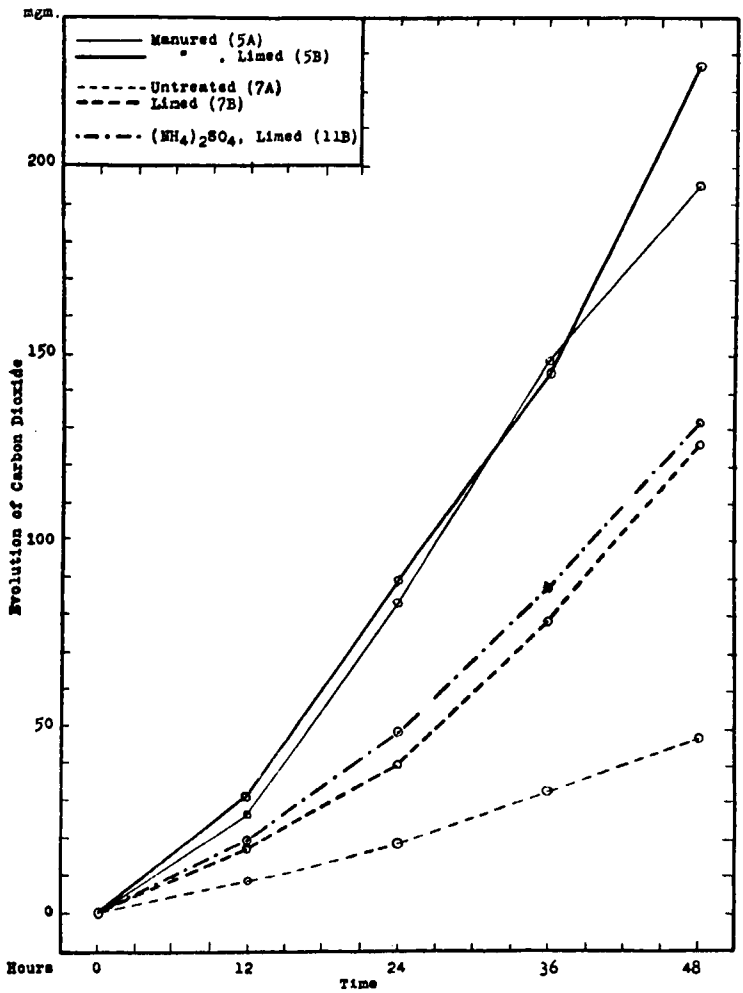

FIG. 2. COURSE OF CARBON DIOXIDE PRODUCTION FROM DEXTROSE ADDED TO SOILS OF DIFFERENT FERTILITY

The addition of lime to a soil which has been cultivated for 15 years, without any additional fertilizers (7B) greatly increased the crop yield. This may be due to the reaction (pH = 6.5) which favors the activity of the nitrogen-fixing bacteria, as will be shown in a subsequent contribution. The reaction also affects the other microbiological activities, the nitrifying and oxidative capacities and especially the number of microörganisms. The comparatively

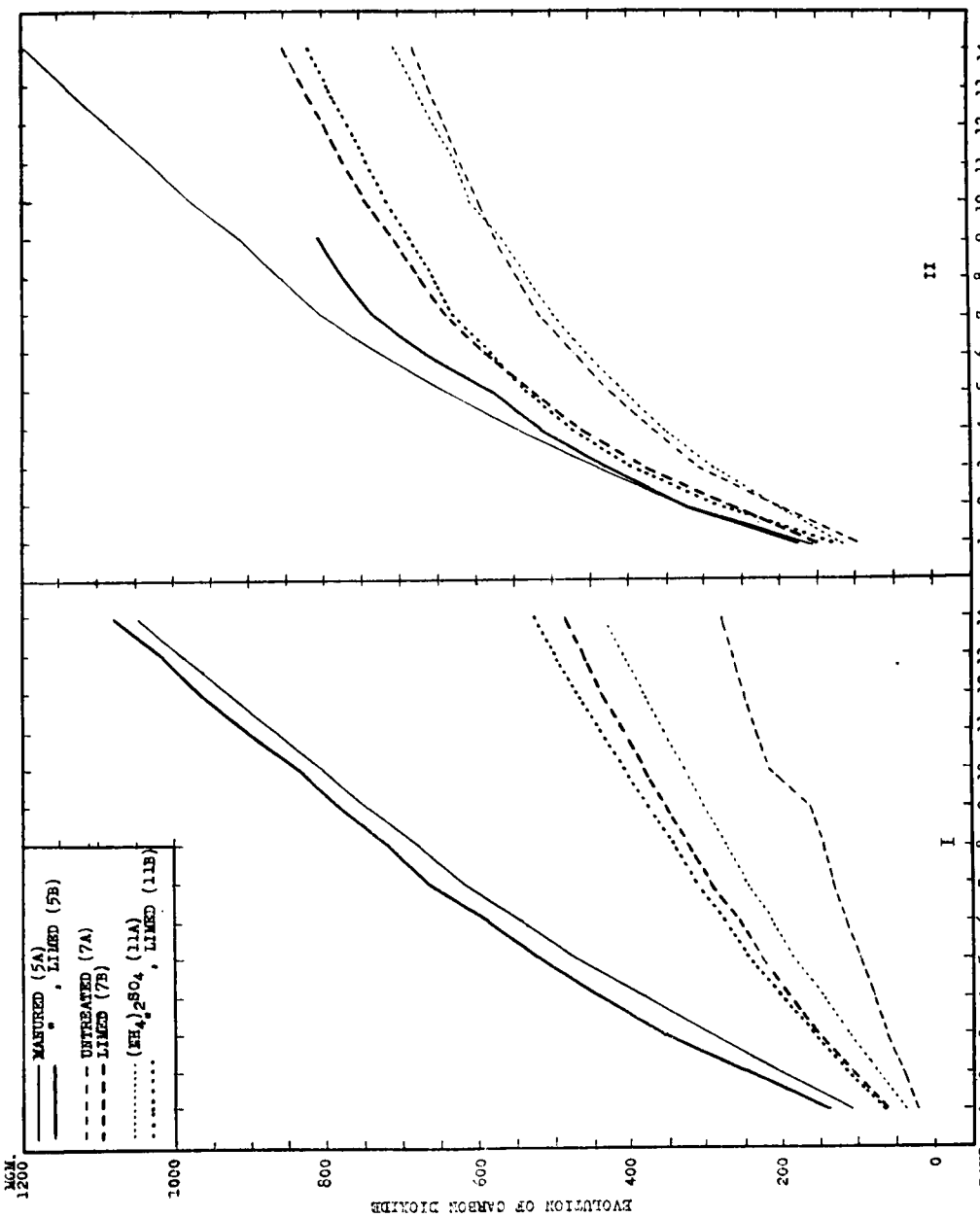

FIG. 3. EVOLUTION OF CARBON DIOXIDE FROM FRESH SOILS OF DIFFERENT FERTILITY WITHOUT FURTHER TREATMENT (I); AND IN PRESENCE OF 0.2 PER CENT ALFALFA MEAL (II)

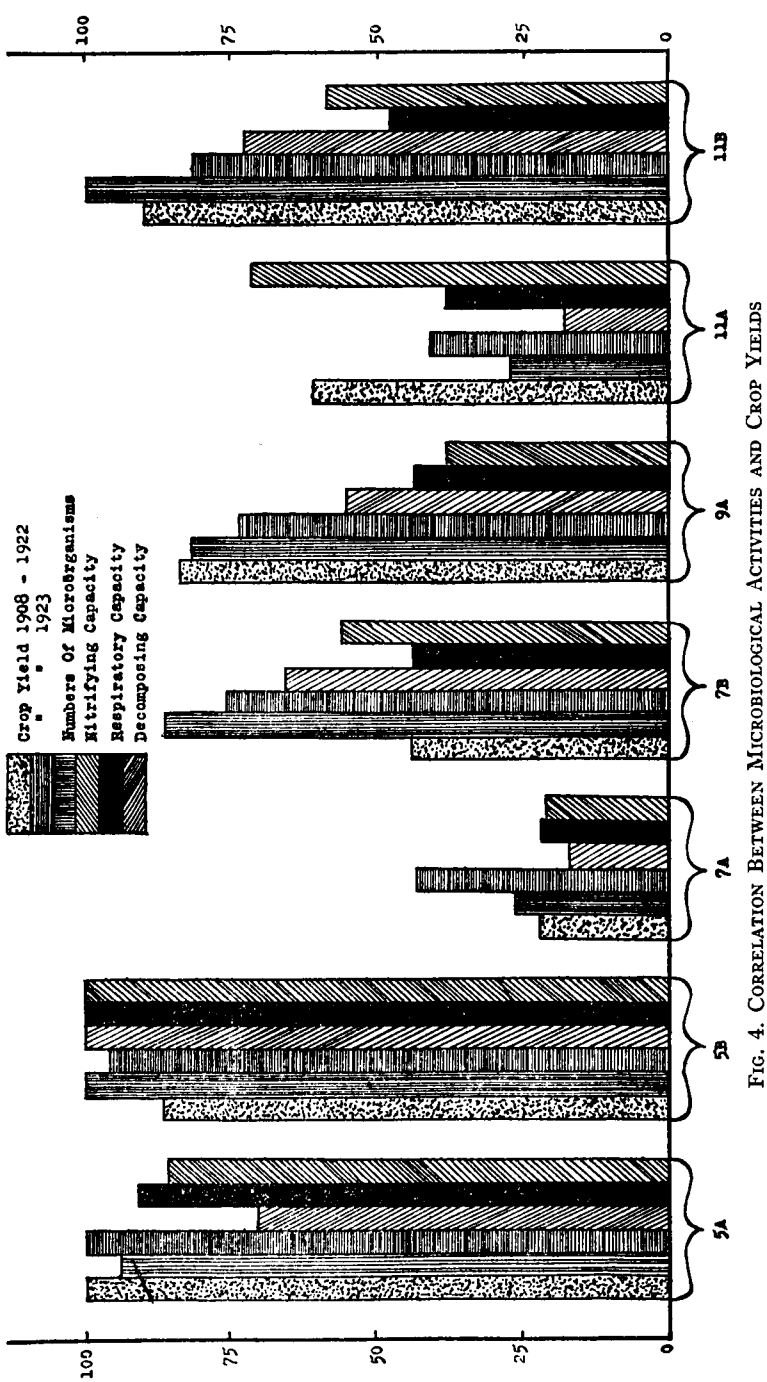

FIG. 4. CORRELATION BETWEEN MICROBIOLOGICAL ACTIVITIES AND CROP YIELDS

low respiratory power of this soil is caused rather by the low content of the available organic matter than by microbiological inactivity. It is interesting to note that although the carbon contents of 7A and 7B are almost the same, the respiratory power, or the amount of carbon dioxide produced by 1 kgm. of soil kept for fourteen days under optimum conditions, is nearly twice as high in 7B than in 7A. This serves also to emphasize the fact that it is not sufficient to determine the respiratory power of a soil as a measure of its capacity to produce carbon dioxide from the viewpoint of microbiological methods, but it is also necessary to determine the relative rapidity with which these solids decompose readily available sources of energy, like dextrose.

The fact that the mere use of inorganic fertilizers, without stable manure, green manure or lime will not work towards the formation of a soil supporting any active microbiological flora is brought out clearly in the results from soil 9A. Although the total crop yield has been kept very high, comparatively low numbers of microörganisms and especially the low nitrifying, respiratory and decomposing powers seem to indicate that the soil is not as active biologically in comparison with the crop yields as are the other soils. The fact that the respiratory and decomposing powers of a soil obtaining artificial fertilizers, as in the case of 9A, are not as high as its crop yield need not necessarily mean that these microbiological activities cannot serve as indices of soil productivity. These two factors—microbiological activity and soil productivity—need not of necessity be related. The one, crop production, is at any moment dependent to a large extent upon the inorganic nutrients present of the soil, while the other, microbiological activity, is regulated to a much larger extent by the abundance of soil organic matter. A soil composed of little else than quartz sand with available elements essential to plant growth may support plants temporarily and still lack any abundant microbial flora. In such cases the *microbiological activities* and *soil productivity* are not correlated but the first *may be better considered as forecasting the future possibilities of the soils.* So with these studies an abundant microbial life may better be considered to indicate that the soil has been built up to a state of fertility which is more permanent than when the microbial activity is considerably less. Although the two may not be correlated at any one time they both approach the same limit.

Similar results are obtained in the case of the soil receiving ammonium sulfate and lime (11B). In this case, the crop yield for 1923 was higher accompanied by a more abundant microbial flora, greater nitrifying and oxidative powers. Since the carbon content is about alike in both plots and the soil reaction is nearly alike, we would expect about the same respiratory powers. As a matter of fact, 11B, with a somewhat lower carbon content, has a somewhat higher respiratory power, corresponding to the better microbiological activities.

Soil 11A is abnormal; the continued use of ammonium sulfate resulted in a great increase in acidity (pH 3.9 to pH 4.4) and an abundant fungous flora.

The added nitrogen is not taken away by the plants and a part of it is probably present as absorption compounds of the zeolitic silicates and a part in the fungus mycelium. The crop yield has been constantly decreasing till in 1923 it is as low as in 7A, the soil receiving no fertilizer. The comparatively low numbers of bacteria, low nitrifying and respiratory powers go hand in hand with the low crop yield. However, the decomposing power of this soil is very high, probably due to the decomposition of the dextrose added, by the abundant fungus flora in the presence of the available nitrogen.

SUMMARY

Determination of the amounts of carbon dioxide evolved from the soils, both without and with the addition of small amounts of organic matter, can be used in grading these soils on the basis of their fertility as well as can determinations of the numbers of microörganisms and nitrification in the soils. The data presented in this paper together with those published previously on the microbiological analysis of soils allow us to look forward to the development of a group of quantitative methods for determining the productive capacity of the soil.

To measure the capacity of the soil to produce carbon dioxide, two methods are suggested: One, determining the amount of carbon dioxide formed from one kilogram of fresh soil, for fourteen days under optimum conditions of temperature and moisture; two, determining the amount of carbon dioxide produced from 500 mgm. of dextrose added to 100 gm. of fresh soil, in forty-eight hours.

Soils rich in organic matter produce by far the greatest amount of carbon dioxide (this does not apply to peats, mucks or such abnormal soils). The amount of carbon dioxide produced is not, however, proportional to the carbon content of the soils. The addition of lime to an acid soil stimulates the production of carbon dioxide, but not to as great an extent as nitrification. This is due to the difference in the nature of the organisms responsible for the chemical changes.

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